Detecting gene mutations in Japanese Alzheimer’s patients by semiconductor sequencing

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ABSTRACT
Alzheimer’s disease (AD) is the most common form of dementia. To date, several genes have been identified as the cause of AD, including PSEN1, PSEN2, and APP. The association between APOE and late-onset AD has also been reported. We here used a bench top next-generation sequencer, which uses an integrated semiconductor device, detects hydrogen ions, and operates at a high-speed using nonoptical technology. We examined 45 Japanese AD patients with positive family histories, and 29 sporadic patients with early onset (<60-year-old). Causative mutations were detected in 5 patients in the familial group (11%). Three patients had a known heterozygous missense mutation in the PSEN1 gene (p.H163R). Two patients from 1 family had a novel heterozygous missense mutation in the PSEN1 gene (p.F386L). In the early onset group, 1 patient carrying homozygous APOE-ε4 had a novel heterozygous missense mutation in the PSEN2 gene (p.T421M). Approximately 43% patients were APOE-ε4 positive in our study. This new sequencing technology is useful for detecting genetic variations in familial AD.

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1. Introduction
Alzheimer’s disease (AD) is a progressive neurodegenerative disease characterized by amnesia, poor judgment, impaired visuospatial abilities or language functions, and changes in personality. (McKhann et al., 2011) Neuronal loss, senile plaques, and neurofibrillary tangles are the pathologic hallmarks of AD. To date, several genes have been identified as the cause of AD, including PSEN1 (MIM 104311) (Sherrington et al., 1995), PSEN2 (MIM 600759) (Levy-Lahad et al., 1995), and APP (MIM 104760) (Goate et al., 1991). These genes are involved in the amyloid-beta pathway, and have been included as causative genes in the new diagnostic guidelines for AD (Jack et al., 2011). PSEN1 mutations are the most frequent, whereas PSEN2 mutations are very rare. The presenilin 1 and 2 proteins are components of the γ-secretase complex. APP encodes the amyloid precursor protein, and mutations are mainly adjacent to the β and γ secretase cleavage site (Cohn-Hokke et al., 2012). APOE (MIM 107741) has also been associated with late onset AD because the frequency of APOE-ε4 allele was found to be high in these patients (Saunders et al., 1993). APOE was proposed to be a moderately penetrant gene with semi-dominant inheritance (Genin et al., 2011).

Until recently, these genes were screened using Sanger sequencing. However, Sanger sequencing is time-consuming and costly; therefore, it is not ideal for routine diagnostic purposes. Over the past few years, high-throughput genome technologies have changed the genetic landscape of AD (Bettens et al., 2013). We used ion-sequencing technology in the present study. Ion sequencing uses an integrated semiconductor device to detect hydrogen ions and operates at high-speed using nonoptical technology. We also assessed the disadvantages of this technology.

2. Methods

2.1. Subjects
Participants were diagnosed with probable AD by neurologists. We tested 45 patients with a positive family history of dementia (familial group, age of onset: 40–82 years). Although most cases showed a dominant inheritance, some patients had incomplete
penetrance. In addition, we enrolled 29 patients with early onset (>60-year-old) AD who had no family history (early onset group, age of onset: 39–59 years). New variations were also analyzed in 112 Japanese control subjects and control exome data were obtained from 147 patients undergoing exome analysis for diseases other than AD (in-house exome). The research procedure was approved by the Ethics Committee of Hiroshima University. All examinations were performed after obtaining written informed consent from the patients or their families.

2.2. Sequencing

Genomic DNA was extracted from the peripheral lymphocytes of the patients. Sixty-seven primers pairs for the exonic regions of PSEN1, PSEN2, APP, and APOE were designed by the Ion AmpliSeq Designer (https://www.ampliseq.com/browse.action) as 2 sub-groups; Primer pool 1 for 35 amplicons and primer pool 2 for 32 amplicons (Supplementary Table 1). Four regions were not covered by originally primer design (Supplementary Table 2), and the uncovered regions are amplified by polymerase chain reaction (PCR) respectively, and sequenced directly by Sanger method.

We performed Ion Personal Genome Machine (PGM) sequencing according to the provided protocol of Ion Ampliseq Library Kit 2.0 (Life Technologies, Carlsbad, CA, USA) and the Ion PGM-200 sequencing supplies kit (Life Technologies). We examined amplicon coverage after ion PGM sequencing. The amplicons were validated with standard PCR-based amplification followed by sequencing analysis using an Applied Biosystems 3130 DNA sequencer (Life Technologies) showed no signs of dementia and did not carry a variant of PSEN1. Two brothers of patient 4 also developed dementia. The elder brother carried the same variant, whereas the younger brother did not agree to the test (Fig. 1). Segregation analysis through the family also provided evidence that this variant was pathogenic.

A novel heterozygous variation of PSEN2 (c.773C>T, p.A258V) was detected, but it was not predicted to be a pathologic variation by any prediction program. One variation in APP (c.1530G>C, p.K510N) was identified in the APP gene, but was detected in both a control subject and in one of our in-house exome databases. Thus, the variation did not appear to be a pathogenic variant.

3. Results

3.1. Amplicon sequencing data

Table 1 shows the total sequencing outputs and mean read lengths from the 2 types of chips. The means of Ion 314 chip and Ion 318 chip based on >20× coverage were 93.9% and 93.7%, respectively. An average 2.62 amplicons per sample of depths of coverage below 20× reads were recognized, and we validated these by Sanger sequencing. The distribution of the mean coverage depth for all exon targets, displayed by amplicon, is shown in Supplementary Table 3. The average read depths of each gene revealed 572.5 in APP, 650.4 in PSEN1, 569.6 in PSEN1, and 425.9 in APOE, respectively. The mean coverage depth of each sample, is shown in Supplementary Table 4. The average read depth was 569.1 (min = 64.5, max = 2462.4).

3.2. Variants in causative genes

We identified 5 heterozygous variations in 4 genes in familial cases (Table 2). Two variants were detected in the PSEN1 gene. One was a known heterogeneous missense mutation in the PSEN1 gene (patient 1, 2, and 3: c.488 A>G, p.H163R, rs53750590). Another was a novel heterozygous missense variant in the PSEN1 gene (patient 4: c.1158C>A, p.F386L, Fig. 1). The variant was determined to be pathogenic by 2 function-prediction algorithms, but was not detected in our in-house exome or the controls. The father was already deceased and had had dementia; the 80-year-old mother showed no signs of dementia and did not carry a variant of PSEN1. Two brothers of patient 4 also developed dementia. The elder brother carried the same variant, whereas the younger brother did not agree to the test (Fig. 1). Segregation analysis through the family also provided evidence that this variant was pathogenic.

APP alleles were quantified using a SYBR Green real-time PCR assay on the Applied Biosystems StepOnePlus System (Life Technologies). We designed probes to detect APP duplication in intron 1, exon 7, and exon 18 of the APP locus (Rovelet-Lecrux et al., 2006). We used the GAPDH as a control gene.

3.3. APP duplication

Using real-time PCR, we confirmed that no patient with AD had a genomic APP duplication.

3.4. Clinical characteristics of patients

The clinical characteristics of the patients are shown in Table 3. All the patients who had gene mutations were early onset (40 to 55-year-old). Patients 2 and 3 were sisters. Patients 4 and 6 were sister and brother. Patient 5 was sporadic. More information on patient characteristics is provided in Table 3.

4. Discussion

We detected a new mutation in the PSEN1 gene (c.1158C>A, p.F386L) and one known mutation in the PSEN1 gene (c.488 A>G, p.H163R, rs83750590). The percentage of detected gene mutations...
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